



Effect of Ibotenic Acid and Colchicine Infusion into the Rat Hippocampus on Retention of Associative Memory

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ABSTRACT

Background: Current models of memory impairment involve the specific brain regions lesion, such as hippocampus, amygdale, nucleus basalis magnocellularis (NBM), and entorhinal cortex. There is some disagreements with respect to the specific roles of these structures, perhaps due to sub regional differences within each area. The aim of the present study was to evaluate the effect of intra-hippocampal administration of ibotenic acid and colchicine on aversive associative memory.

Methods: Forty male Wistar rats were used in this study. Animals were cannulated bilaterally in the CA1 and the dentate gyrus regions of the hippocampus for infusion of ibotenic acid and colchicine (8 $\mu\text{g}/\mu\text{L}$), respectively. Then step-down passive-avoidance learning (PAL) was used 14 days post injection. Data were analyzed using independent T-test, and a p value $<.05$ was considered as statistically significant.

Results: Intra-dentate gyrus administration of 8 $\mu\text{g}/\mu\text{L}$ colchicine caused no significant change in PAL ($p > .05$). Also, intra CA1 administration of ibotenic acid demonstrated no significant change in memory retention tested 24 h after training compared to the control groups ($p > .05$).

Conclusion: This study findings showed that single dose of colchicine or ibotenic acid targeting CA1 and dentate gyrus of hippocampus caused no significant change in associative memory in passive avoidance learning paradigm.

Keywords: Hippocampus, Lesion, Colchicine, Ibotenic acid

Introduction

Neurodegenerative and neurotraumatic diseases such as Alzheimer's disease (AD) and brain trauma and stroke result in cognitive deficits [1]. Although extensive research has been carried out on cognitive therapies, but these efforts have not yet yielded successful results in restoring learning and memory capability due to the complexity of the subject. Memory formation involves information encoding, storing, and recalling [2], which requires the cooperation of the neural network.

Various brain regions, including amygdala, hippocampus, and cortical areas, are part of the neural network that subserves passive avoidance. Several studies have shown that passive avoidance depends on hippocampal function and its NMDA receptors. Infusion of NMDA receptors antagonist, AP5, into the dorsal hippocampus of mice profoundly impairs passive avoidance retention [3]. Similarly, neurotoxic lesions of the corticohippocampal circuitry (perirhinal, postrhinal, and entorhinal cortices) cause profound deficits in passive avoidance learning in rats [4]. Hippocampus has been known to play a pivotal role in encoding, consolidating, and retrieving the associative memories [5, 6]. The traditional view of hippocampal-dependent consolidation asserts that newly acquired information is reliant on the hippocampus for some time after a learning episode, during which hippocampal circuitry facilitates the gradual establishment of long-term memories in a distributed neocortical network [7]. It has long been known that damage to the hippocampal system, including dentate gyrus, CA1, and CA3, results in retrograde and anterograde amnesia [8], as it is evident in patients with Alzheimer's disease [9].

However, there are inconsistencies in the literature. For example, hippocampal damage has been reported to facilitate avoidance learning in shuttle box [10, 11]. Also, damage to GABAergic neurons in the medial septum (a major non-cortical input to the hippocampus)

prior to avoidance training causes no change in the acquisition of avoidance responses [12].

Ibotenic acid acts as an glutamate receptors agonist (preferentially binds to NMDA receptors) and exerts its toxic effect by prolonged activation of these receptors, resulting in increased influx of chloride and calcium ions, the entry of excess water, and osmotic lysis of the cell [13].

Direct administration of colchicine into the hippocampus of rats results in the preferential destruction of dentate gyrus granule cells without affecting the surrounding pyramidal cells. Injection of colchicine into other brain areas also destroys neurons but with less selectivity than that observed in the hippocampus. The neurotoxicity of colchicine appears to be related to its ability to bind to tubulin [14].

Considering the importance of animal research to understand mechanisms involved in neurodegenerative disorders, particularly AD, and the need to find new therapies, this study aimed to evaluate the effects of chemical lesions on the performance in passive avoidance learning. Passive avoidance task has been widely used to evaluate the effects of drug administration, lesions, and behavioral manipulations on cognitive functions such as long-term associative memory [15]. For this reason, the hippocampus was chosen as the target for inducing lesions in the present study. In order to clarify the roles of this area in acquisition and retention, ibotenic acid and colchicine were bilaterally injected into the CA1 and the dentate gyrus regions of the hippocampus. To assess learning and memory, step-down passive avoidance task was employed.

Methods

Animals

Forty male Wistar rats with a mean age of 3 months and a mean weight of 220 g were

obtained from our laboratory breeding stock and used in this study. The rats were housed in groups of 5 in each cage and kept under controlled temperature conditions (22 °C) and light/darkness cycles of 12 h /12 h (lights on at 8:00 a.m.). Food and water were available ad libitum. The rats were tested during the first half of the light cycle. The investigations were carried out according to the NIH publication No: 8023, revised in 1978. Approval code was Research council 85.5.25.

Stereotaxic surgery

Before surgery, the animals were randomly distributed into four groups and received 1) bilateral hippocampal CA1 lesion (n=10), 2) bilateral dentate gyrus lesion (n=10), and 3, 4) saline for each toxin (n=20). All the animals were subjected to stereotaxic surgery under general anesthesia (IP) with a dose of 60 mg/kg ketamine (Netherland) and 4 mg/kg xylazine (Bayer, Leverkusen, Germany).

The cannulas were targeted to the hippocampus with the incisor bar set at -2.7 mm below the interaural line according to the following coordinates from the stereotaxic atlas for the CA1 region (AP: -3 mm from bregma, L: \pm 2 mm from midline, and V: -2.8 mm) and for the dentate gyrus region (AP: -2.7 mm, L: \pm 2.1, and V: -3.4 [16] from the skull surface).

Experiment 1

The aim of this experiment was to evaluate the effects of bilateral hippocampal lesions induced by colchicine prior to learning on the acquisition and long-term retention in step-down passive avoidance conditioning. The animals were gently restrained by hand; the stylets were removed from the guide cannulas and replaced by 27-gauge injection needles (1 mm below the tip of the guide cannula), which were connected by polyethylene tubing to 10- μ L Hamilton microsyringes. The injection solutions were administered in a total volume of 2 μ L/rat (8 μ g/ μ L) (colchicine, SIGMA, USA). Injection needles were left in place for an additional 60 s to facilitate drug delivery.

Experiment 2

Ten Wistar rats received bilateral ibotenic acid lesions prior to training and were trained in avoidance conditioning. Ibotenic acid (2 μ L/rat,

8 μ g/ μ L) was dissolved in saline and bilaterally injected into CA1. Control groups received the same volume of saline.

Training

After recovery from the surgery, the animals were handled for 3 consecutive days 5 min per day. This was carried out to familiarize the rats with the learning environment and to reduce both the number of pseudo-avoidance responses as well as the inverse relationship between shock intensity and performance because of fear.

A single trial step-down passive-avoidance task was used. This task was performed in a box with 40 \times 30 \times 40 cm dimensions and a floor consisting of parallel 3.0-mm stainless steel bars spaced 1.0 cm apart. The animal was placed on a Plexiglass platform (12 \times 10 \times 7 height cm) in the center of the apparatus. Each rat was gently placed on the platform, then after stepping down from the platform, a single-trial electric foot shock (0.5 mA, 100Hz, cut off 5s) was applied to the grid. When the animal stepped back on the platform, it was immediately withdrawn from the training apparatus. This training procedure was carried out between 9: 00 and 11: 00. During the training period, animals could avoid shock by jumping on the platform.

Retention test

Twenty four hour after the training, each rat was again placed on the platform without any shock delivery. The step-down latency was considered as a measure of retention. The time of descending from the platform and the total time spent on the platform were recorded. Upper cut-off time was set at 300 s [17].

Cannula verification

After the end of the behavioral procedure, all animals were deeply anesthetized, and 1 μ L of a 4% methylene-blue solution was bilaterally infused into the hippocampus (0.5 μ L/ 1 side), then they were decapitated, and the brains were dissected and stored in formaldehyde (10%) for histological evaluation of cannula placements. Then the brains were sliced, and the sites of injections were verified according to Paxinos & Watson (2007) [16] (Fig. 1).

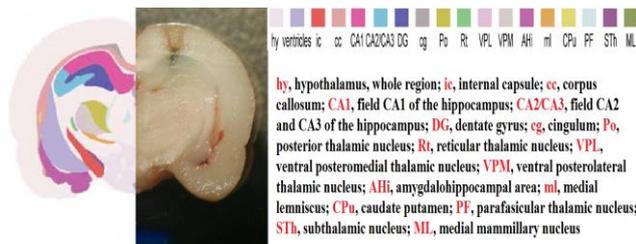


Fig. 1. Rat brain section showing cannulas verification and the extension of the area reached by infusions into the hippocampus.

Data analyses

The normality of data was assessed by Kolmogorov-Smirnov test, and then data were analyzed by student t-test using SPSS software (Ver. 16). Data were presented as means \pm SD, and a *p* value < .05 was considered as significant.

Results

A: The effect of hippocampal ibotenic acid lesion on memory retention

There was no significant difference between the ibotenic acid-lesioned and control groups in terms of initial training latency (6 \pm 0.29 s vs. 5.5 \pm 0.2 s; *p* > .05) as well as retention time (150 \pm 24 s vs. 178 \pm 15 s; *p* > .05) (n=10 in each group) (Fig. 2).

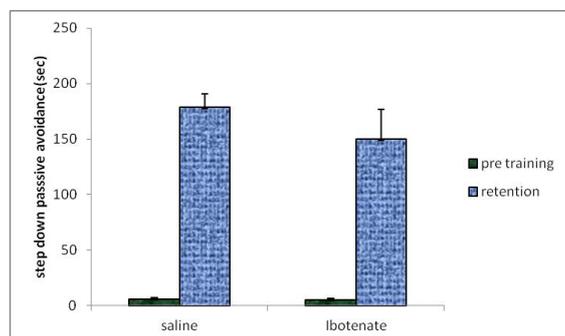


Fig. 2. Influence of ibotenic acid lesion of the CA1 region of hippocampus on passive avoidance response in rats (Mean \pm SD, n=20)

B: The effect of dentate gyrus-selective colchicine lesion on memory retention

No significant change was found by bilateral injection of colchicine neither in training latency (5.5 \pm 0.25 s vs. 4 \pm 0.01) nor in retention test (138.5 \pm 10 s vs. 165.38 \pm 10 s) 24 hours after

learning compared to the control group (*p* > 0.05) (n=10 in each group) (Fig. 3).

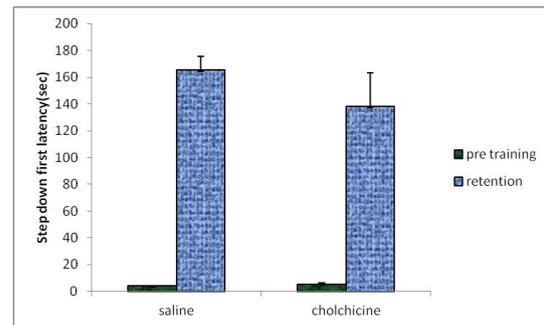


Fig. 3. Influence of colchicine lesion of the dentate gyrus of hippocampus on passive avoidance response in rats (Mean \pm SD, n=20).

Discussion

The main result of the present study was that colchicine infusion into the dentate gyrus of the hippocampus prior to learning did not alter the long-term retention of a passive avoidance task. No significant change was observed in the acquisition of the passive avoidance task compared to the memory impairment previously found after bilateral injection of ibotenic acid into the other areas of the brain, such as entorhinal cortex [18] and nucleus basalis magnocellularis (NBM) [19].

Studies have revealed that ibotenic acid lesion results in a striking loss of acetylcholinesterase (AChE) in the lesioned Nbm [20].

Although no significant memory impairment was found in rats, a slight insignificant detrimental effect on the retention could not be ruled out. On the other hand, lesioned rats did not show any deficits in the acquisition session, indicating that rats were able to form associations and learn by conditioning. Thus, it seems that the rats didn't have deficits in attention, and the slight decrease in avoidance performance might reflect difficulty in retrieving this association.

Post-lesion escape latencies were similar to those in the control group, ruling out nonspecific effects such as hypoactivity; thus, changes in motor activity produced by the lesions could not be accounted for retention.

The present study results are inconsistent with the results of previous experiments [21-23], indicating that rats lesioned with kainate or

colchicine were not able to learn by aversive conditioning. In line with the present study findings, Mundy et al. (1990) made bilateral lesions by injecting ibotenic acid (IBO) into the nucleus basalis magnocellularis (NBM). When compared to controls, rats with lesions in NBM had significantly impaired choice accuracy in the T-maze and radial-maze tasks and significantly performed fewer trials to reach criterion in the acquisition, while having no problem in the retention of an active avoidance task and significantly performing more trials to reach criterion in the passive avoidance task. According to the results, equivalent behavioral changes due to NBM lesions were obtained in tasks that vary in the type of motivation, reinforcement, and response-reinforcement contingency [14].

Ineffectiveness of hippocampal ibotenic acid lesion in the passive avoidance acquisition may be due to lower sensitivity of step-down passive-avoidance paradigm to hippocampus lesions than other memory tasks, and it might be necessary to choose relatively demanding tasks to detect learning deficits in animals with hippocampus lesions.

Regarding the second part of the experiment, in contrast with the previous findings [24,25], colchicine infusion into the dentate gyrus caused no significant memory impairment. The probable reason might be the difference in the site of colchicine administration as they injected colchicine into the hippocampal areas [24,25] and the lateral ventricles [26]. Another possible explanation for the discrepancies could be related to the demanding tasks. For example, Gilberto F. Xavier (2009) used multiple-site colchicine injections (7 µg/µL) throughout the dentate gyrus and assessed the memory by Morris water maze task and reported spatial memory impairment [25].

Besides the specificity of neurons for various memories, the sensitivity of sensory afferents to aversive stimuli provided in the test environment ranges from tactile and visual cues to nociceptive pain and olfactory cues, which involve processing in higher brain centers, including the thalamus (Baarendse et al. 2008). Meanwhile, it should be noticed that selective lesions of the

ventral hippocampus reduces the conditioned freezing responses [27]. However, lesion of the dorsal part of hippocampus in line with our study does not lead to aversive memory impairment.

On the other hand, amygdala, particularly its basolateral nucleus (BLA), is selectively involved in taste aversion acquisition [28, 29] and reversible lesion studies [30, 31].

The amygdala has been known to be essential for aversive learning [32, 33]. Particularly among its subnuclei, the basolateral nucleus plays a major role in the convergence of CS and US for associative learning. The basolateral nucleus of the amygdala is connected to the central nucleus of the amygdala. Outputs from the central nucleus of the amygdala are essentially responsible for the expression of fear responses. This network triggers behavioral adjustments indicative of learning and memory through motor control and concomitant autonomic and endocrine adjustments via different pathways.

Therefore, chemical lesion studies should be more accurately addressed considering neurotransmitters and neural connections. Here, more specific memory assessing tasks, such as Morris water maze and radial or Y maze, are suggested to be employed to detect learning deficits in animals treated by these reagents when the target is hippocampus.

Conclusion

These results indicate that bilateral injections of either ibotenic acid or colchicine into the hippocampus don't provide a reliable model for investigating cognitive dysfunctions when passive avoidance paradigm is used.

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